

# Role of endoscopic tumor tattooing in improving lymph node retrieval of colorectal cancer

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## Background and study aims

Adequate lymphadenectomy is crucial to ensure accuracy in staging and treatment of colorectal cancer. Endoscopic preoperative tattooing to mark the location of the tumor has recently been proposed to promote the recovery of lymph nodes in colorectal specimens. The purpose of the study was to evaluate the role of preoperative endoscopic tumor tattooing in improving the retrieval of lymph nodes in colorectal resections.

## Patients and methods

The study was conducted on 100 candidates randomized into two groups. The first group included 50 patients who were injected with black eye dye by colonoscopy done 3–10 days before surgery (tattooed group), and the 50 other patients in the second group (control group) did not have preoperative tattooing. All patients underwent the standard resection under National Comprehensive Cancer Network Colorectal Cancers Guideline.

## Results

The tattooed group showed higher number of lymph nodes retrieved compared with the control group in colon cases and rectum cases. In eight cases, among the patients included in our study, extra-anatomical lymph nodes (outside the standard resection margins) were detected stained. Of these eight cases, two showed malignant deposits.

## Conclusion

Preoperative endoscopic tattooing of colorectal cancers is a safe and easy technique, which proved to have a benefit in improving lymph node retrieval for resected specimens of colorectal cancers either in obese or nonobese patients and also whether they had received or not a preoperative neoadjuvant therapy. There is a connection between lymphatics draining different parts of the colon, as proved by the presence of tattooed lymph nodes outside the standard resection margins. Its clinical significance needs more studies on a larger sample size.

## Keywords:

colorectal cancer, lymph node, tattooing

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## Introduction

Colorectal carcinoma is the most common malignancy of the gastrointestinal tract [1]. Surgery is the main choice of treatment for patients with colorectal cancer [2]. Consensus recommendations prescribe the evaluation of at least 12 lymph nodes for appropriate staging and for the effective use of postoperative adjuvant therapy. Chemotherapy should be mandatory for patients with node-negative disease and fewer than 12 nodes in the surgical specimen [3,4].

Adequate lymphadenectomy and adequate recovery of the lymph node from the resected specimen are critical to ensuring staging accuracy, particularly to prevent tumor cells from underdiagnosing lymph node involvement. The number of lymph nodes retrieved will depend on various factors like surgical radicality, dedicated pathologist, and patient/tumor-specific

factors [5]. Endoscopic preoperative tattooing to mark the location of the tumor has recently been suggested to promote the retrieval of lymph nodes in colorectal specimens [6,7]. The purpose of this research was to investigate the relationship between ink tattooing performed preoperatively to mark the site of the tumor and lymph nodes retrieval by the pathologist after colorectal resection for cancer.

## Patients and methods

This is a prospective randomized control study that included 100 patients with colorectal cancer, in Cairo

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University Hospital (Kasr Al Ainy), in the period between May 2016 and March 2018. This study was approved by the Ethics Committee of the Kasr Al Ainy Hospital and Cairo University.

The study included all patients with colorectal cancer with age between 18 and 70 years old who fulfilled the following inclusion criteria: TNM staging III TNM and a single colorectal cancer confirmed by colonoscopy biopsy which will undergo radical resection. Patients with remote metastases, previous abdominal cancer surgery, history of other abdominal malignancies, leakage, obstruction, or perforation emergencies were excluded from the study. Patients were randomized using closed envelope technique into two groups: group 1, which included patients who have preoperative endoscopic tattooing, and group 2, which included patients who did not have preoperative tattooing. Informed consent was obtained from each patient. Patients in the group 1 were injected with black eye dye (Fig. 1) during colonoscopy. The injection was done in the submucosa, 2 cm from the lesion proximally and distally at 3, 6, 9, 12 o'clock positions, and in the lesion itself, the injection was done 3–10 days before surgery.

All patients were operated upon, either by open or laparoscopic surgery, by the same team of surgeons and underwent the standard resection according to the recommendations and guidelines of the NCCN for colorectal cancers (National Comprehensive Cancer Network) (Fig. 2).

Any lymph node stained with the dye and laid outside the standard resection margins (in the mesentery of

other parts of the colon) was excised and examined separately (Fig. 3a and b).

Patients with rectal cancers underwent total mesorectal excision of every pelvic lateral lymph node stained with the dye, which was excised and examined separately for deposits. The specimens from all patients were examined by two pathologists independently for all samples, with calculation of total number of lymph node retrieved and the percentage of tattooed lymph node that are positive for malignancy.

The pathological reports of all specimens were collected, and the following data were collected for statistical analysis: age, sex, BMI, site, pathology, size of the specimen (cm), volume of the mass (in  $\text{cm}^3$ ), approach of surgery (open or laparoscopy), preoperative neoadjuvant therapy, T stage of the mass, total number of lymph nodes, and number of positive lymph nodes.

#### Statistical analysis

In terms of mean, SD, median and range, or frequencies (number of cases) and percentages, data were statistically defined where appropriate. Comparison of numerical variables was performed using Mann–Whitney  $U$  test for independent samples between the study classes.  $\chi^2$  test was performed for comparing categorical results. Exact test was used instead when the frequency is predicted to be less than 5. Normal distribution data were expressed as mean  $\pm$  standard deviation (SD) and compared using  $t$  test.

Categorical variables were expressed as percentages and compared, as needed, with the  $\chi^2$  or Fisher's exact test.

Figure 1



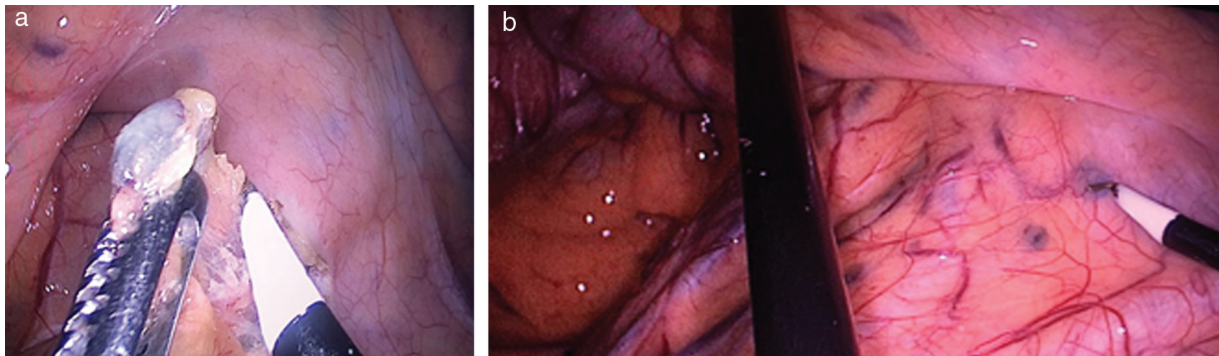
Black eye dye.

Figure 2



Right colon specimen tattooed after resection.

Figure 3



(a and b) Tattooed lymph nodes as seen during laparoscopy.

All *P* values were two sided, and the statistically significant *P* value was found to be less than 0.05. Statistical analysis was conducted with version 25.0 of SPSS software (SPSS Inc., Chicago, Illinois, USA).

## Results

The study included 100 patients, divided into two groups: the tattooed group ( $n=50$ ) and the control group ( $n=50$ ), as shown in Table 1. Both groups were matched regarding sex (46% M vs. 58% M,  $P=0.23$ ), age [55 (40, 64), vs. 57 (41, 65)  $P=0.532$ ], and BMI [29 (26, 33) vs. 28 (25, 32)  $P=0.882$ ] (Figs 4 and 5). There was no statistically significant difference regarding the site of the tumor between the two groups (Fig. 5), the size of the specimens, volume of the masses, and neoadjuvant therapy [21 (42%) vs. 20 (40%),  $P=1$ ].

As shown in Table 2, the frequency of open approach was higher in the control group compared with the

tattooed group [34 (68%) vs. 18 (36%),  $P=0.003$ ]. However, there was no significant difference between laparoscopic and open approaches in the total number of harvested lymph nodes (16 vs. 14,  $P=0.19$ )

As shown in Table 3, the tattooed group showed higher number of harvested lymph nodes compared with the control group in colon cases (21 vs. 15,  $P=0.009$ ), rectum cases (16 vs. 10,  $P=0.019$ ), elderly patients (20 vs. 15,  $P=0.005$ ), obese patients (15 vs. 10,  $P=0.044$ ), nonobese patients (20 vs. 15,  $P=0.01$ ), open approach (15 vs. 10,  $P=0.018$ ), laparoscopic approach (22 vs. 16,  $P=0.001$ ), patients of neoadjuvant chemotherapy (15 vs. 10,  $P=0.012$ ), and patients without neoadjuvant chemotherapy (22 vs. 16,  $P=0.009$ ).

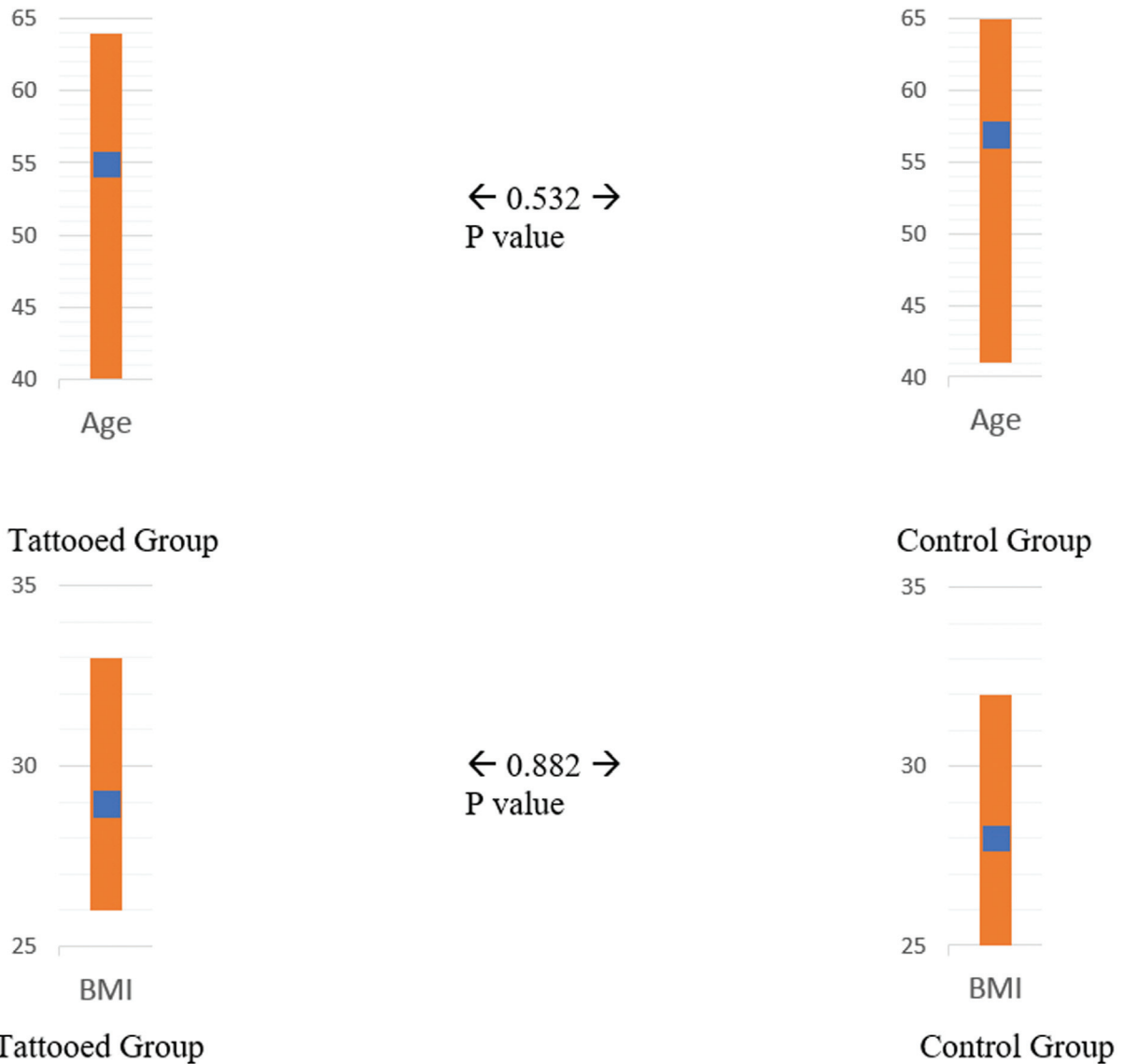
During operations, there were eight cases in which extra-anatomical tattooed lymph nodes were detected; six of these cases were negative for malignancy, whereas two cases were positive. One positive case was a case of

**Table 1 Demographic data and baseline characteristics**

	Tattooed group (n=50)	Control group (n=50)	P value
Age (years)	55	57	0.532
Male sex	46%	58%	0.23
BMI (kg/m <sup>2</sup> )	29 (26, 33)	28 (25, 32)	0.882
Site			0.69
Colon	50%	56%	
Rectum	50%	44%	

Data are presented as median (quartiles) and percentages.

**Figure 4**



Age and BMI of both groups. Data are presented as median (quartiles).

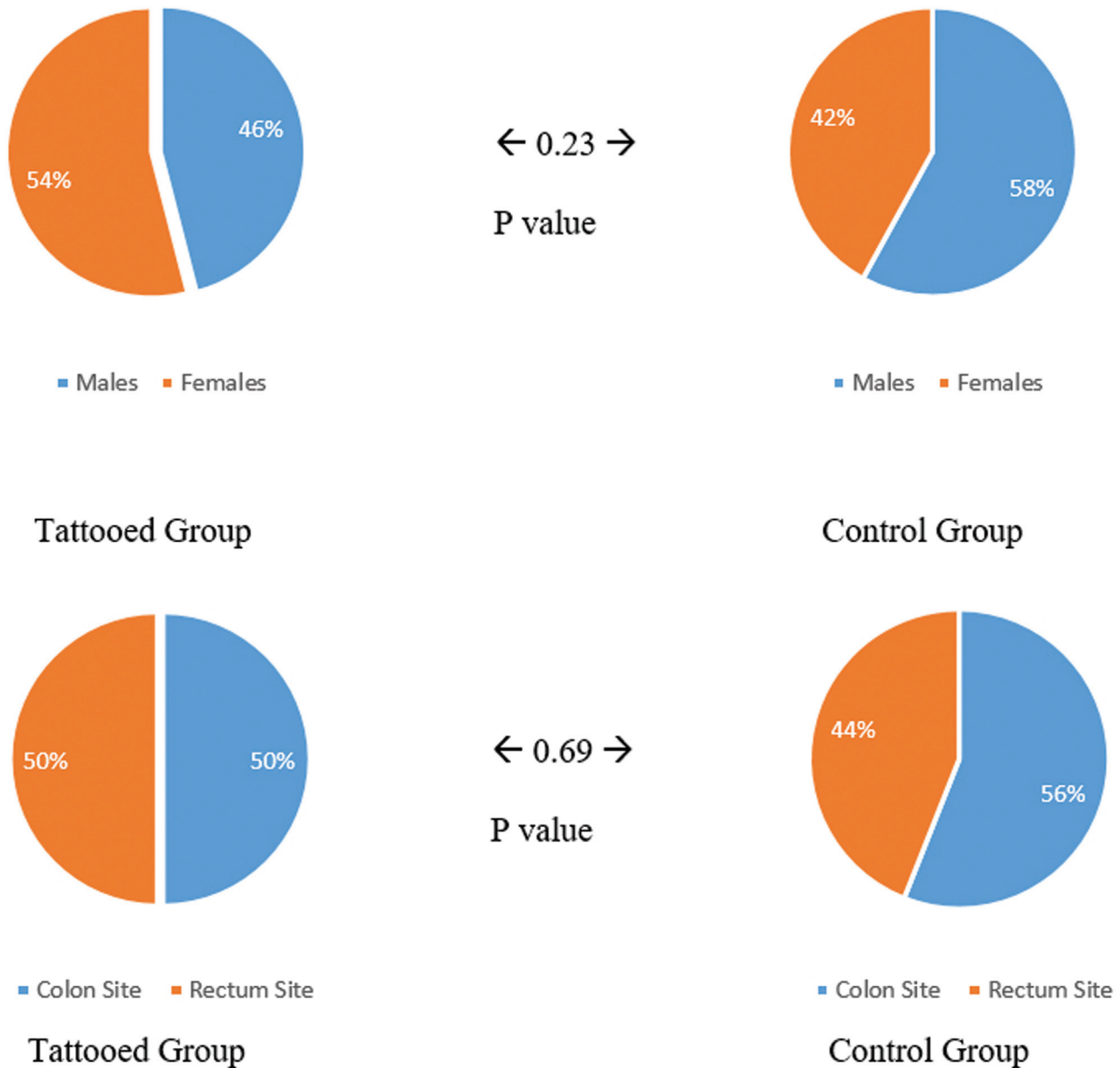
metastatic cancer cecum, for which right hemicolectomy was done. A single tattooed lymph node at the root of the middle colic was found, and histopathological examination was positive also for metastasis which was only discovered during surgery (intraoperative finding). There was a liver deposit in the left lobe of the liver. The other positive case was a case of sigmoid mass, for which sigmoidectomy was done. Two tattooed

lymph nodes were found along the course of superior mesenteric artery, and histopathological examination was positive for metastasis.

### Discussion

Identifying the progression of the neoplastic disease to the lymph nodes is of the utmost importance to assess

Figure 5



Sex and tumor distribution. Data are presented as percentages.

both the prognosis and the appropriate care in patients with colorectal cancer [8]. In addition, a higher number of sampled lymph nodes has emerged in several previous studies as an independent prognostic factor for improved survival, particularly in stage II CRC [9]. Originally, endoscopic tattooing was introduced as a reliable and precise method for localizing colonic lesions on follow-up colonoscopies, that is, incompletely resected polyps or scars from prior polypectomies. Especially after the introduction of laparoscopic and robotic surgery in colorectal tumors resection, the use of the endoscopic tattooing has been used widely to determine the exact site for tumor resection [7].

Recently, the use of ink tattooing to identify the tumor site during preoperative colonoscopy has been reported to improve the quality of pathology testing by raising the number of lymph nodes obtained and adequate lymphadenectomy in colorectal cancer specimens [8]. However, several factors that influence the assessment of the existence of neoplastic disease in lymph nodes as well as the total lymph nodes are present; these factors include patient and tumor characteristics, surgeon, and pathologist. All these were taken into consideration during the present study.

In our study, we used the endoscopic tattooing in improving the total number of lymph nodes

**Table 2 Surgical and pathological data**

	Tattooed group (n=50)	Control group (n=50)	P value
Size of the specimen (cm)	25 (18, 35)	27 (18, 36)	0.804
Volume of the mass (cm <sup>3</sup> )	20 (10, 42)	20 (12, 37)	0.863
Pathology			0.65
Adenocarcinoma Gill	76%	70%	
Mucinous adenocarcinoma	24%	30%	
Surgical approach			0.003
Open	36%	68%	
laparoscopic	64%	32%	
Neoadjuvant therapy	42%	40%	1

Data are presented as median (quartiles) and percentages.

**Table 3 Total lymph nodes**

	Tattooed group (n=50)	Control group (n=50)	P value
Total lymph nodes	19	12	0.001
Subgroup (Site)			
Colon	21	15	0.009
Rectum	16	10	0.019
Subgroup (age)			
Elderly	19	10	0.095
Nonelderly	20	15	0.005
Subgroup (BMI)			
Obese	15	10	0.044
Nonobese	20	15	0.01
Subgroup (neoadjuvant chemotherapy)			
Yes	15	10	0.012
No	22	16	0.009
Subgroup (surgical approach)			
Open	15	10	0.018
Laparoscopic	22	16	0.001

Data are presented as median values.

harvested from the specimen. We compared the total number of lymph node harvested from both groups (control and tattooed). There was a significant increase in total number of lymph node harvested from tattooed group than the control group ( $P=0.001$ ), in patients with colon and rectum cancer.

Tattooing also increased the number of harvested lymph node in most of the subgroups (obese population, non-obese population, and patients who received neoadjuvant chemotherapy prior to surgery as well as those who went immediately for cancer resection).

In eight cases, among the patients included in our study, extra-anatomical lymph nodes (outside the standard resection margins) were detected stained. The presence of these tattooed lymph nodes outside the standard resection margins consolidates the theories which stated that there is a connection between lymphatic vessels in the mesentery itself. Of the eight tattooed lymph nodes, six detected outside the standard

resection margins were negative, and the remaining two were positive. One of these positive nodes was detected at the root of the middle colic artery in a case of metastatic cancer cecum. Its prognostic and clinical significance is questionable, especially as there were multiple metastases in the liver (discovered only intraoperatively).

The second node was detected along the course of the superior mesenteric artery in a case of sigmoid cancer. The clinical significance of such a case still needs more study on a large-sample size to detect its importance and its role in recurrence and if it will change the standard management plan or not. Regarding the rectal cancer cases, many authors, especially in the Far East school recommend extended lymphadenectomy as a routine in low rectal cancers [10].

Another recent study done by Georgiou *et al.* [11], to assess the value of extended (lateral) lymphadenectomy in the operative management of locally advanced and recurrent rectal cancer concluded that lateral pelvic sidewall lymphadenectomy does not pose a

statistically relevant benefit regarding oncological and perioperative outcomes in such patients.

We found only three cases with tattooed lymph nodes on the lateral pelvic wall, which were all negative for metastasis. However, we need to conduct the study on a larger sample size to detect the efficacy of endoscopic tattooing in selecting cases that are in need of lateral pelvic wall dissection not only total mesorectal excision.

There are various drawbacks to the current research. First, our research is a single-center, small sample research and may not be applicable to the general population.

Second, our research did not examine long-term follow-up data, including recurrence of disease and survival free of disease. We cannot therefore directly evaluate the relation between tattooing of the lymph nodes and prognosis.

Large-sample, prospective, multicenter studies and further investigations with longer duration are needed.

### Conclusion

Preoperative endoscopic tattooing significantly increases total number of lymph nodes retrieved from the specimens, in colon or rectum specimens, obese and nonobese patient and whether the patient received preoperative chemoradiation or not. There is a connection between lymphatics draining different parts of the colon, as proved by presence of tattooed lymph nodes outside the standard resection margins. Its clinical significance needs more studies on a larger sample size. The use of preoperative endoscopic tattooing in selecting cases who are candidates for lateral pelvic wall dissection, not only total mesorectal excision, needs further studies on a superior sample size.

### Conclusion

In conclusion, our study findings show that preoperative endoscopic tumor tattooing is a safe

and efficient technique that could be used in colorectal surgery to increase lymph nodes harvesting from specimens, and accordingly, enhancing staging and proper treatment. Hence, in hospitals that include colorectal surgery, we suggest performing preoperative endoscopic tumor tattooing procedure.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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